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# Impact of Baseline Retinal Nonperfusion and Macular Retinal Capillary Nonperfusion on Outcomes in the COPERNICUS and GALILEO Studies

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***Purpose:*** To evaluate the impact of baseline retinal capillary nonperfusion (RNP) and macular retinal capillary nonperfusion (MNP) status on outcomes at week 24 (W24).

***Design:*** Post hoc analyses of 2 phase 3, randomized, double-masked, multicenter, sham-controlled studies.

***Participants:*** Three hundred sixty-six patients with macular edema secondary to central retinal vein occlusion randomized in COPERNICUS and GALILEO.

***Methods:*** We randomized patients 3:2 to receive intravitreal aflibercept 2 mg every 4 weeks or sham injections until W24. RNP and MNP were assessed by a masked independent reading center.

***Main Outcome Measures:*** Proportion of patients with 10 disc areas (DA) or more of RNP and any degree of MNP at W24, relative risks of 10 DA or more of RNP or any degree of MNP at W24 developing, change from baseline in best-corrected visual acuity (BCVA) and central retinal thickness (CRT) by baseline RNP and MNP status, and relationship between baseline RNP and MNP status.

***Results:*** At baseline, 24.6% of patients showed 10 DA or more of RNP and 72.6% showed MNP, regardless of baseline RNP status. At W24, the pooled proportions of patients in the intravitreal aflibercept and sham groups with 10 DA or more of RNP were 11.6% and 29.0%, respectively ($P = 0.0001$); the respective proportions with any degree of MNP were 61.2% and 79.5% ($P = 0.0008$). Relative risks and 95% confidence intervals for intravitreal aflibercept versus sham were 0.4 (0.25−0.62) for 10 DA or more of RNP and 0.8 (0.68−0.90) for MNP, indicating a lower risk for these outcomes with intravitreal aflibercept than with sham. Mean BCVA change was greater in intravitreal aflibercept- versus sham-treated eyes, with less than 10 DA and 10 DA or more of RNP at baseline (+17.5 vs. +0.8 letters and +18.3 vs. −4.1 letters, respectively) and with and without baseline MNP (+15.7 vs. +0.3 letters and +17.1 vs. +0.4 letters, respectively). Agreement between baseline RNP and MNP status was low ($\kappa = 0.12$). The proportions of patients with 1 or more ocular serious adverse event in the intravitreal aflibercept- and sham-treated groups, respectively, were 3.2% and 11.3%.

***Conclusions:*** At W24, visual and anatomic improvements, including perfusion status, were greater in eyes treated with intravitreal aflibercept than in eyes treated with sham, regardless of baseline RNP or MNP status. *Ophthalmology Retina 2019;3:553-560 © 2019 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Retinal vein occlusion (RVO) affects approximately 16.4 million adults worldwide; of those, 2.5 million experience central retinal vein occlusion (CRVO),[1] and the 15-year incidence rate of CRVO is estimated to be 0.2%.[2] Macular edema secondary to CRVO is the leading cause of vision loss in these patients.

Macular edema is the result of increased vascular permeability and breakdown of the blood−retinal barrier, secondary to hypoxia-related upregulation and release of vascular endothelial growth factor (VEGF), making it an important treatment target.[3] Intravitreal aflibercept is a fusion protein of key domains from human VEGF receptors 1 and 2 with the constant region of human immunoglobulin G that binds to VEGF, notably VEGF-A, and placental growth factor.[4] The efficacy and safety of intravitreal aflibercept in the treatment of patients with macular edema secondary to CRVO have been demonstrated in the pivotal Controlled Phase 3 Evaluation of Repeated intravitreal administration of VEGF Trap-Eye in Central retinal vein occlusion: Utility and Safety (COPERNICUS)[5−7] and General Assessment Limiting Infiltration of Exudates in central retinal Vein Occlusion with EYLEA (GALILEO)[8−10] studies. Although most patients in both studies demonstrated perfused retinas (i.e., <10 disc areas [DA] of retinal capillary nonperfusion



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[RNP] at baseline; 76.5% of patients in the intravitreal aflibercept group and 73.8% in the sham group), patients with RNP of 10 DA or more represented a considerable proportion of the total population in these studies.[11] At week 24 in the COPERNICUS study, baseline RNP status did not affect visual outcomes, with gains in best-corrected visual acuity (BCVA) of +17.8 versus −2.3 Early Treatment Diabetic Retinopathy Study (ETDRS) letters for patients with RNP of 10 DA or more and +17.1 versus −4.8 letters for patients with RNP of less than 10 DA receiving intravitreal aflibercept versus sham, respectively. Furthermore, anatomic outcomes were not affected by perfusion status at baseline.[5] Outcomes for baseline RNP status in the GALILEO study at 24 weeks were not reported,[8] although the proportion of patients who had perfused retinas was similar to that of the COPERNICUS study.[5] Baseline macular retinal capillary nonperfusion (MNP) status has not been reported previously for these studies.

Capillary nonperfusion is an important clinical feature of RVO that may impact its clinical course.[12] In clinical research, this is assessed by measurement of RNP on 7 standard field conventional fluorescein angiography (FA), which has an angle of 30-35 degrees. The Central Vein Occlusion Study (CVOS)[13] divided patients into those with less than 10 DA and those with 10 DA or more of RNP; the latter group was classified as *ischemic*. In addition to this imaging-based definition of ischemia proposed by CVOS, and to cover more comprehensively the functional impact of retinal nonperfusion, Hayreh and colleagues suggested a combination of 4 functional tests (visual acuity, visual fields, relative afferent pupillary defect [RAPD], and electroretinography) and 2 morphologic tests (ophthalmoscopy and FA)[14]; however, this approach lacks full clinical validation.

In this article, we report week 24 outcomes in COPERNICUS and GALILEO in relation to baseline RNP and MNP status. We also determined the association between these different approaches to evaluating ischemia in CRVO.

## Methods

### Study Design

The details of these studies have been published previously.[5–11] Herein, we describe key aspects of study design and conduct. COPERNICUS (clinicaltrials.gov identifier, NCT00943072) and GALILEO (clinicaltrials.gov identifier, NCT01012973) were parallel, randomized double-masked phase 3 studies comparing intravitreal aflibercept with sham for the treatment of macular edema secondary to CRVO. The studies were conducted in 124 sites in the United States, Canada, South America, Europe, Asia, and Australia. Institutional review board or ethics committee approval was obtained at each site. The studies were conducted in compliance with ethical guidelines from the tenets of the Declaration of Helsinki and the International Conference on Harmonization. Institutional review board or ethics committee approval was obtained at each clinical study site before the studies commenced, and all patients provided written informed consent.

### Participants

Adult patients with center-involved macular edema resulting from CRVO of 9 months' duration or less were included if central retinal thickness (CRT) was 250 μm or more on time-domain OCT and BCVA was 73 to 24 ETDRS letters in the study eye. Only 1 eye per patient was included. We report post hoc analysis results for the integrated dataset of the 2 studies. Retinal capillary nonperfusion of 10 DA or more was not an exclusion criterion. Exclusion criteria are reported elsewhere.[5–10]

### Randomization and Treatments

In COPERNICUS and GALILEO, patients were randomized 3:2 to receive intravitreal aflibercept 2 mg every 4 weeks or sham injections until week 24. From week 24 until week 52, all intravitreal aflibercept-treated patients in both studies and the sham-treated patients in COPERNICUS were eligible to receive intravitreal aflibercept based on predefined visual and anatomic retreatment criteria. Given this difference, the post hoc analyses reported here are limited to week 24.

### Outcomes

The primary end point in both COPERNICUS and GALILEO was the proportion of eyes that gained 15 letters or more in BCVA at week 24. Results for the primary end point of these studies are reported elsewhere.[5,8]

The end points of the current post hoc analysis were mean changes in BCVA and CRT at week 24 based on subgroups of patients with less than 10 and 10 DA or more of RNP at baseline, and with and without any degree of MNP at baseline. We also assessed the relationship between RNP and MNP status at baseline.

In these post hoc analyses, we classified eyes as having less than 10 DA or 10 DA or more of RNP on FA anywhere in the standard fundus 7 subfields as scored by the masked central reading center (Digital Angiography Reading Center, Great Neck, NY). All FA images were assessed by 3 independent readers masked to study treatment. In case of disagreement on categorical assessments, the third senior reader had the final judgement on the result to be entered into the clinical database. Intraobserver and interobserver variability in determining areas of nonperfusion were not measured. For numerical values, the mean of the readers' assessments was calculated. Assessments were made using quadrants and subfields as defined by a modified ETDRS grid,[15] with areas of nonmacular capillary nonperfusion traced manually in each quadrant. Total areas of nonperfusion were quantified by the addition of areas of nonperfusion in all 4 quadrants. We included eyes classified as indeterminate at baseline and at week 24 in the group with 10 DA or more of RNP for this analysis if nonperfusion could not be differentiated from other imaging features, because generally these were patients with severe disease, often with extensive retinal hemorrhages. We also classified eyes as having any degree of MNP in 1 or more locations (i.e., in any area) of the ETDRS grid centered on the macula as scored by the central reading center at baseline and at week 24.

### Statistical Analysis

We pooled week 24 data for each treatment group in COPERNICUS and GALILEO. Descriptive statistics are provided for all outcomes for less than 10 DA and 10 DA or more of RNP and any or no degree of MNP at baseline. The relative risks for 10 DA or more of RNP and any MNP at week 24 were analyzed using the Mantel-Haenszel method adjusted for study, from which the ratio in proportions of patients between treatment groups and a corresponding 95% confidence interval were estimated. Because there was no heterogeneity based on the test, the Mantel-Haenszel relative risk method for assessing treatment effects (sham vs. intravitreal aflibercept) is valid. For postbaseline analyses, missing values were replaced by using the last observation carried forward

Table 1. Mean Baseline Best-Corrected Visual Acuity and Central Retinal Thickness by Baseline Retinal Capillary Nonperfusion and Macular Retinal Capillary Nonperfusion Status

| | Intravitreal Aflibercept | | | | Sham | | | |
|---|---|---|---|---|---|---|---|---|
| | *<10 Disc Areas of Retinal Capillary Nonperfusion* | *≥10 Disc Areas of Retinal Capillary Nonperfusion* | *Macular Retinal Capillary Nonperfusion* | *No Macular Retinal Capillary Nonperfusion* | *<10 Disc Areas of Retinal Capillary Nonperfusion* | *≥10 Disc Areas of Retinal Capillary Nonperfusion* | *Macular Retinal Capillary Nonperfusion* | *No Macular Retinal Capillary Nonperfusion* |
| No. | 166 | 51 | 123 | 45 | 104 | 37 | 81 | 32 |
| BCVA (ETDRS letters) | 54.9 | 42.7 | 53.2 | 54.5 | 52.0 | 44.0 | 49.2 | 56.6 |
| CRT (μm) | 666.2 | 691.7 | 649.5 | 658.7 | 614.0 | 777.0 | 671.5 | 605.5 |

BCVA = best-corrected visual acuity; CRT = central retinal thickness; ETDRS = Early Treatment Diabetic Retinopathy Study.

approach. We assessed the association between eyes with 10 DA or more of RNP anywhere in the retina and eyes with any degree of MNP at baseline using the κ coefficient. Descriptive statistics are provided for BCVA and CRT changes over time. For analyses of RNP and MNP, we replaced missing values by the last observed postbaseline value.

## Results

### Efficacy

At baseline, 23.5% of patients in the intravitreal aflibercept group and 26.2% of patients in the sham group showed 10 DA or more of RNP, and 73.2% of intravitreal aflibercept-treated patients and 71.7% of sham-treated patients showed MNP. Baseline BCVA and CRT by treatment group and baseline RNP and MNP status are shown in Table 1.

The validity of pooling the results of the COPERNICUS and GALILEO studies was confirmed by the Q statistic, because heterogeneity was not indicated ($P = 0.3479$; Table 2). Mantel-Haenszel relative risk for 10 DA or more of RNP at week 24 of 0.4 (95% confidence interval, 0.25−0.62) indicated lower risk for intravitreal aflibercept compared with sham treatment. Pooled proportions of eyes with 10 DA or more of RNP at week 24 in the intravitreal aflibercept group versus the sham group were 11.6% versus 29.0% ($P = 0.0001$).

Validity of the pooled MNP analysis was confirmed, because heterogeneity was not indicated by Q statistic ($P = 0.2222$; Table 2). Mantel-Haenszel relative risk for MNP at week 24 of 0.8 (95% confidence interval, 0.68−0.90) indicated a lower risk for intravitreal aflibercept compared with sham treatment. Pooled proportions of eyes with MNP at week 24 in the intravitreal aflibercept group versus the sham group were 61.2% versus 79.5% ($P = 0.0008$).

The treatment effects at week 24 with respect to mean changes in BCVA from baseline were consistent regardless of baseline RNP status or MNP status for intravitreal aflibercept (Fig 1A). Mean change in BCVA at week 24 was +17.5 letters in intravitreal aflibercept-treated eyes with less than 10 DA of RNP at baseline and +18.3 letters in intravitreal aflibercept-treated eyes with 10 DA or more of RNP at baseline. Mean change in BCVA at week 24 was +15.7 letters in intravitreal aflibercept-treated eyes with MNP at baseline and +17.1 letters in intravitreal aflibercept-treated eyes without MNP at baseline. Sham-treated eyes with 10 DA or more of RNP at baseline showed greater declines in vision through week 24 (−4.1 letters vs. +0.8 letters in eyes with less than 10 DA of RNP at baseline; Fig 1B).

Intravitreal aflibercept rapidly reduced CRT regardless of baseline RNP or MNP status (Fig 2A). In intravitreal aflibercept-treated eyes, mean CRT reduction was −441.0 μm in those with less than 10 DA of RNP at baseline and −493.8 μm in those with 10 DA or more of RNP at baseline. Similarly, mean CRT reduction in intravitreal aflibercept-treated eyes was −423.1 μm in the group with MNP at baseline and −448.5 μm in those without MNP at baseline. Among the sham-treated patients, those with 10 DA or more of RNP at baseline showed the highest CRT at baseline (777.0 μm; Table 1) and the greatest decrease in CRT at week 24 (−264.3 μm; Fig 2B).

Of the 358 eyes that were randomized in COPERNICUS and GALILEO, 281 with nonmissing values for RNP and MNP were

Table 2. Relative Risk for Retinal Capillary Nonperfusion and Macular Retinal Capillary Nonperfusion at Week 24

| | Relative Risk for ≥10 Disc Areas of Retinal Capillary Nonperfusion at Week 24 | | | | Relative Risk for Macular Retinal Capillary Nonperfusion at Week 24 | | | |
|---|---|---|---|---|---|---|---|---|
| | *Intravitreal Aflibercept* | *Sham* | *Relative Risk (95% Confidence Interval)* | *Heterogeneity* | *Intravitreal Aflibercept* | *Sham* | *Relative Risk (95% Confidence Interval)* | *Heterogeneity* |
| COPERNICUS | 16/110 (14.5) | 27/63 (42.9) | 0.3 (0.20–0.58) | | 46/93 (49.5) | 38/54 (70.4) | 0.7 (0.54–0.92) | |
| GALILEO | 8/97 (8.2) | 9/61 (14.8) | 0.6 (0.23–1.37) | | 63/85 (74.1) | 51/58 (87.9) | 0.8 (0.72–0.99) | |
| Pooled | 24/207 (11.6) | 36/124 (29.0) | 0.4 (0.25–0.62) | Chi-square, 0.88 | 109/178 (61.2) | 89/112 (79.5) | 0.8 (0.68–0.90) | Chi-square, 1.49 |
| *P* value | | | 0.0001 | 0.3479 | | | 0.0008 | 0.2222 |

Data are no. (%) unless otherwise indicated.

considered for the κ coefficient analysis. Fluorescein angiography imaging at baseline showed that 51 eyes demonstrated 10 DA or more of RNP and 230 eyes demonstrated less than 10 DA of RNP. Of eyes with 10 DA or more of RNP, 48 (94.1%) demonstrated MNP and 3 (5.9%) demonstrated no MNP. Of the eyes with less than 10 DA of RNP, 156 (67.8%) demonstrated MNP and 74 (32.2%) demonstrated no MNP. Distribution of RNP and MNP status was balanced between the intravitreal aflibercept and sham groups. Macular retinal capillary nonperfusion was observed in 72.6% of eyes at baseline. Because almost all eyes with 10 DA or more of RNP demonstrated MNP and approximately two thirds of eyes with less than 10 DA of RNP demonstrated MNP, there was low agreement between baseline RNP status and baseline MNP status ($\kappa = 0.12$).

### Safety (Full Population)

At week 24, the proportions of patients with 1 or more ocular serious adverse event in the intravitreal aflibercept-treated and sham-treated groups, respectively, were 3.2% and 11.3%. In the intravitreal aflibercept group, iris neovascularization, macular ischemia, retinal artery occlusion, visual acuity reduced, vitreous detachment, endophthalmitis, and corneal abrasion each occurred in 1 patient.

There were 2 Anti-Platelet Trialists' Collaboration-defined arterial thromboembolic events in sham-treated patients in COPERNICUS (1 fatal arrhythmia and 1 fatal myocardial infarction), and there were none in GALILEO.

## Discussion

These post hoc analyses demonstrated that, in intravitreal aflibercept-treated patients, visual and anatomic gains were rapid and similar regardless of baseline RNP or baseline MNP status. Similar features included rapid improvement after treatment initiation, early plateau, and maintenance of visual acuity gains. Patients with 10 DA or more of RNP at baseline generally showed worse baseline BCVA but demonstrated positive outcomes similar to those seen in patients with less than 10 DA of RNP at baseline. Mean change in CRT was greater in the intravitreal aflibercept group than the sham group whether eyes had less than 10 DA or 10 DA or more of RNP at baseline and whether they had any degree of MNP at baseline or none. There were no differences in visual or anatomic outcomes when the 16 indeterminate patients (intravitreal aflibercept, n = 6; sham, n = 10) were removed from the RNP subgroup (data not shown), suggesting that the treatment effects observed were not influenced by this subset of patients. The proportions of eyes with 10 DA or more of RNP and with any degree of MNP decreased from baseline to week 24 in intravitreal aflibercept-treated eyes and increased in sham-treated eyes and were significantly different between the 2 treatment groups at week 24.

The proportion of patients with 10 DA or more of RNP and with MNP at week 24 was lower in the intravitreal aflibercept group than in the sham group ($P = 0.0001$ and $P = 0.0008$, respectively). These results support the conclusion that treatment with intravitreal aflibercept in these CRVO trials may be beneficial, potentially improving

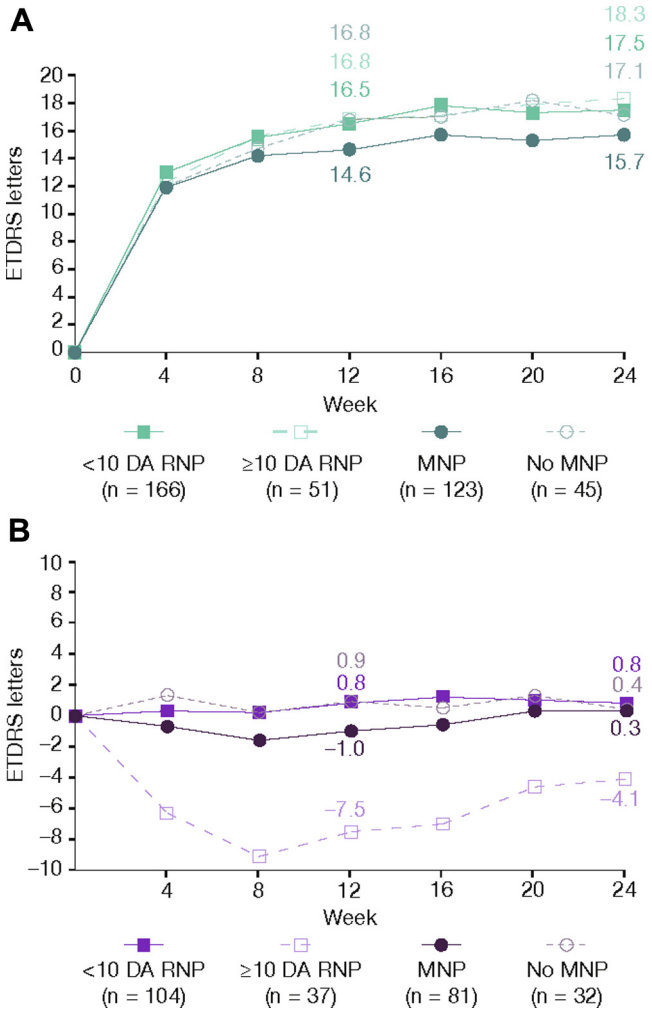


**Figure 1.** Graph showing the mean change in best-corrected visual acuity (BCVA) by baseline retinal capillary nonperfusion (RNP) and baseline macular retinal capillary nonperfusion (MNP) status for patients in the (**A**) intravitreal aflibercept and (**B**) sham treatment groups. Retinal capillary nonperfusion includes indeterminate eyes (COPERNICUS: intravitreal aflibercept, n = 4; sham, n = 7; and GALILEO: intravitreal aflibercept, n = 2; sham, n = 3). DA = disc area; ETDRS = Early Treatment Diabetic Retinopathy Study.

perfusion of retinal capillaries that are malfunctioning but not destroyed.

Most patients in the COPERNICUS and GALILEO studies demonstrated MNP at baseline, regardless of their baseline RNP status. Statistically, the high prevalence of MNP at baseline results in the low agreement found with the κ analysis (κ = 0.12). Because a large proportion of patients with MNP (approximately 70%) also were perfused (less than 10 DA RNP) at baseline, this explains in part the lack of agreement between MNP and RNP, which also may involve peripheral retinal nonperfused areas that contribute to ischemic disease severity. Because any degree of nonperfusion of the macula was characterized as nonperfused, this designation does not seem to provide a meaningful discrimination of ischemic disease severity in RVO.

For those patients with 10 DA or more of RNP at baseline, nearly all (approximately 94%) also demonstrated MNP at baseline, suggesting that those with 10 DA or more of RNP have MNP and more nonperfusion throughout the retina. Retinal capillary nonperfusion involves larger parts of the retina morphologically. Macular retinal capillary nonperfusion may be a factor related to central visual impairment at baseline in CRVO but may not correlate directly to the total extent of visual impairment. Therefore, consideration must be given to RNP in the periphery, as well as throughout the retina, in assessing disease severity.

Because anti-VEGF therapy provides anatomic improvement in all eyes as early as week 1, this implies that every eye demonstrating CRVO at presentation harbors ischemia or nonperfusion to some extent, which leads to continued VEGF production.[16] However, the term *ischemia* (which is undoubtedly at a cellular level) should not be used interchangeably with *nonperfusion*, which is an FA determination of flow through retinal vascular beds. Some of this nonperfusion may be caused by leukocyte aggregation secondary to VEGF in capillaries that are still viable[17] and potentially could be improved by VEGF blockade. If nonperfusion is sufficiently severe in localized areas, then retinal atrophy occurs with death of neurosensory cells, including capillary beds.[18] Vascular endothelial growth factor suppression would be unlikely to result in revascularization of such avascular areas. This study demonstrated that most patients with CRVO and macular edema have measurable areas of capillary nonperfusion on FA (either MNP, RNP outside of the macula, or both) that in general improves with intravitreal aflibercept therapy. A recent post hoc analysis from the RIDE and RISE phase 3 studies of ranibizumab in eyes with diabetic macular edema (DME) and MNP showed that eyes with concurrent DME and baseline MNP, as well as those without baseline MNP, showed visual and anatomic improvement with ranibizumab treatment compared with sham for up to 24 months, despite lower BCVA and increased CST at baseline in those with baseline MNP.[19] It should be noted that the proportion of patients with baseline MNP in RIDE and RISE was lower (25%–28%) than in our study (approximately 70%).

Complications of ischemic RVO disease include posterior neovascularization with vitreous hemorrhage or anterior segment neovascularization with secondary neovascular glaucoma. In the Rubeosis Anti-VEGF Trial,[20] eyes with severe CRVO showed visual and anatomic improvements after treatment with ranibizumab; however, despite these improvements, the risk of neovascular complications was delayed but not eliminated. The Rubeosis Anti-VEGF Trial investigators proposed that the term *preproliferative* should be used regarding patients previously described by Hayreh[20] as *ischemic*. In COPERNICUS and GALILEO, the number of patients who demonstrated neovascularization was very low (n = 11 patients). At week 24, neovascularization was observed more frequently in eyes with 10 DA or more of RNP than in those with less than 10 DA of RNP and in those with MNP than in those without MNP, regardless of treatment group (data not shown). However, given the small

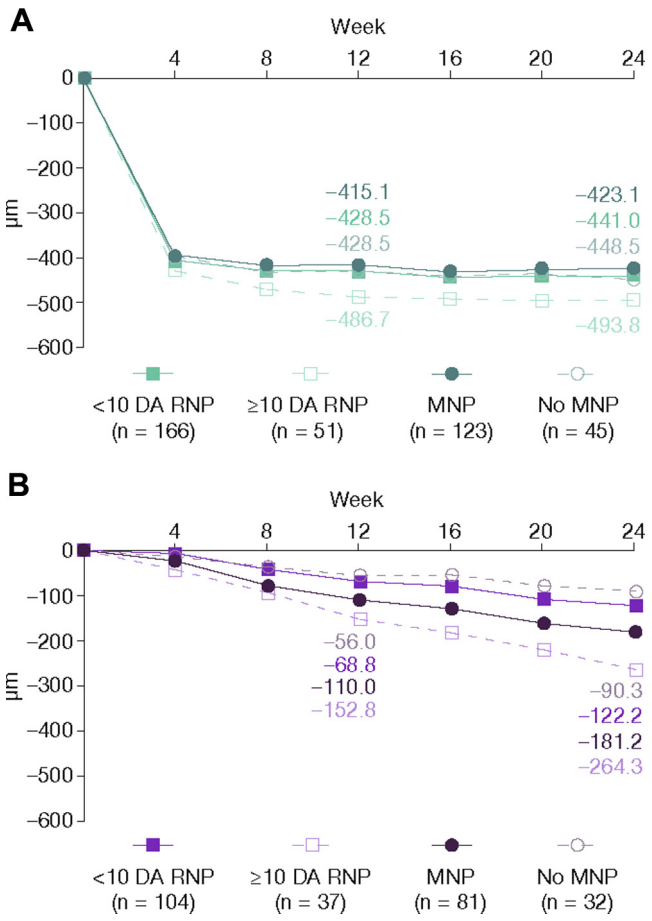


**Figure 2.** Graphs showing mean change in central retinal thickness by baseline retinal capillary nonperfusion (RNP) and baseline macular retinal capillary nonperfusion (MNP) status for patients in the (**A**) intravitreal aflibercept and (**B**) sham treatment groups. Retinal capillary nonperfusion includes indeterminate eyes (COPERNICUS: intravitreal aflibercept, n = 4; sham, n = 7; and GALILEO: intravitreal aflibercept, n = 2; sham, n = 3). DA = disc area.

numbers in these subgroups, it is not possible to draw any firm conclusions regarding the influence of RNP or MNP status on neovascularization.

Even in clinical trials, there is no universal approach for identifying and evaluating ischemic patients as a basis for inclusion or exclusion criteria. This lack of a standardized system for identifying nonperfusion poses challenges for a comparison across studies. The Ranibizumab for the Treatment of Macular Edema after Central Retinal Vein Occlusion Study: Evaluation of Efficacy and Safety (CRUISE)[21] and CRYSTAL[22] studies excluded patients based on the presence of RAPD (a sensitive test for differentiating preproliferative CRVO), which is associated with marked levels of capillary nonperfusion in both the macula and the periphery.[23] As acknowledged by the authors of the CRUISE study, this exclusion criterion "may have effectively eliminated patients with extensive capillary dropout."[21] In contrast, COPERNICUS and GALILEO were the first trials of anti-VEGF agents for the treatment of RVO that did not exclude patients based on RAPD. Therefore, the results of these studies may be more generalizable to a broader patient population with regard to nonperfusion or preproliferative status, although there were relatively few eyes with 10 DA or more of RNP at baseline (approximately 18%–20%).

Assessments of ischemia in RVO disease have constraints in sensitivity and specificity of diagnosis because of the various limitations of technologies previously used to categorize ischemic disease. Standard FA using the 7 subfields view to diagnose RNP was used by the Central Vein Occlusion Study more than 30 years ago. Although this test has limited prediction for progression to ischemic complications, based on the current post hoc analyses, RNP (10 DA or more) provides, at a minimum, a larger area than MNP (including the macula and some peripheral retina) to assess ischemic status. In contrast, the results of the current analyses suggest that the MNP assessment, which is based on any degree of RNP within the ETDRS grid focused on the macula, may have even lower sensitivity and specificity. Furthermore, in a population of patients for whom RAPD was used to exclude those with ischemic RVO, MNP diagnosis, in theory, would have even weaker predictive strength to identify those patients with CRVO who are at risk of progression to neovascular complications.

Strengths of the present study include the use of 3 masked graders from a central reading center to evaluate FA images and determine baseline RNP and MNP status (with the third senior reviewer having the final judgment on the results to be entered into the database in the case of disagreement), as well as the strict protocols of these well-designed randomized clinical studies.

There are limitations and weaknesses to our study. The primary end point of the 2 trials was the 24-week data point, and because of differences in treatment regimens between the trials, was the maximum time point for our assessment. However, results from the individual longer-term findings of COPERNICUS and GALILEO show that improvements in BCVA and anatomic changes are maintained for up to 52 weeks in patients continuing the same treatment regimen.[6,9] Published results of the GALILEO study show that the improvements in BCVA with intravitreal aflibercept in perfused (<10 DA) and nonperfused (≥10 DA) eyes at week 24 are maintained at week 52. In addition, for the sham-treated cohorts, improvements in BCVA in perfused (<10 DA) eyes at week 24 were maintained at week 52, whereas the decline in BCVA in nonperfused (≥10 DA) eyes over 24 weeks was maintained at week 52.[9]

The use of 7-standard field FA in COPERNICUS and GALILEO may be viewed as a methodologic limitation. Although once considered the historical gold standard to identify RNP in RVO,[24] 7-standard field FA has been surpassed by superior methods to assess RNP that were not available when our study was undertaken. However, in their post hoc analysis, Reddy et al[19] used the same 7-standard field FA assessment method and showed beneficial effects of anti-VEGF therapy in patients with MNP at baseline. Another limitation was that 7-standard field FA may be inadequate at baseline in at least one third of patients because of masking from extensive retinal hemorrhages or poor images resulting from media opacities.[14] The coexistence of extensive macular edema could lead

systematically to an underestimation of the nonperfused area by leakage of dye. However, because this is a potential systematic methodologic bias, this may have impacted the results for both intravitreal aflibercept and sham-treated eyes equally. Variability also may be introduced because of patient fixation per visit. OCT angiography, which has been used to evaluate macular nonperfusion,[25,26] and widefield FA, which provides up to 200° imaging of the retina[27] and has become more common in clinical practice, were not available when these studies started. However, it should be noted that widefield FA does not allow for circumventing of hemorrhages and opacities, and quantitative analyses remain challenging. As a result, access to the central venous vasculature also is limited with this method. Newer widefield swept-source OCT angiography may provide much greater understanding of the role and progression of capillary bed damage in future CRVO studies, without the need for intravenous fluorescein.

Although the proportion of patients with missing data (77 of 358 patients [21.5%]) initially may seem to be a limitation, it should be noted that this proportion is lower than that reported in the CRYSTAL study (38.7%).[22] It is likely that proportions of patients with missing data in this range are typical for studies using this specific analytical technique.

In conclusion, these post hoc analyses of COPERNICUS and GALILEO demonstrate the benefits of intravitreal aflibercept in macular edema resulting from CRVO regardless of baseline RNP or baseline MNP status. Future studies of the morphologic effects of anti-VEGF therapy using widefield FA studies, coupled with advanced imaging technologies such as newer widefield OCT angiography imaging, may provide further clarity regarding the scope of treatment benefit possible in ischemic RVO.

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Abbreviations and Acronyms:
**BCVA** = best-corrected visual acuity; **COPERNICUS** = Controlled Phase 3 Evaluation of Repeated intravitreal administration of VEGF Trap-Eye in Central retinal vein occlusion: Utility and Safety; **COVS** = Central Vein Occlusion Study; **CRT** = central retinal thickness; **CRVO** = central retinal vein occlusion; **DA** = disc area; **DME** = diabetic macular edema; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **FA** = fluorescein angiography; **GALILEO** = General Assessment Limiting Infiltration of Exudates in central retinal Vein Occlusion with EYLEA; **MNP** = macular retinal capillary nonperfusion; **RAPD** = relative afferent pupillary defect; **RNP** = retinal capillary nonperfusion; **RVO** = retinal vein occlusion; **VEGF** = vascular endothelial growth factor.

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